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Epigenetic Dysregulation through Histone Modifications in Lymphoma

Poruchy epigenetické regulace na úrovni modifikací histonů u lymfomů

Bakalářská práce

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Poděkování

Rád bych poděkoval především svému školiteli, doc. MUDr Ondřeji Havránkovi, Ph.D., za jeho vstřícnost a pomoc, bez kterých bych práci nemohl napsat. Také děkuji mým kamarádům a rodičům, kteří mě podporovali během celého studia.

Prohlášení

Prohlašuji, že jsem tuto závěrečnou práci zpracoval samostatně z uvedené literatury a na základě konzultací se svým školitelem. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

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Abstrakt

Lymfomy jsou různorodá skupina nádorů, které vznikají z lymfocytů a často se tvoří v lymfatických uzlinách nebo ve slezině. Lymfomy jednak patří mezi jedny z nejčastějších druhů zhoubných nádorů ve světě, a spousta typů má špatnou prognózu anebo není zatím možné jejich úplné vyléčení.

U některých typů lymfomů, stejně tak jako u spousty jiných druhů nádorových onemocnění, jsou velmi časté mutace enzymů, které se funkčně podílejí na post-translačních modifikacích histonových proteinů. Tyto enzymy přímo ovlivňují genovou expresi tím, že mění kondenzaci a tím i přístupnost chromatinu. Vzhledem k tomu, že některé z těchto enzymů hrají důležitou roli při vzniku zárodečných center v lymfoidních folikulech, jejich mutace mohou vést k nekontrolované proliferaci a vzniku nádorového onemocnění. Standardní léčebné přístupy pomocí chemo-imunoterapie nejsou u podstatné části lymfomů dostatečné k jejich vyléčení. Nové a více cílené postupy s případnou inhibicí nebo modulací funkce těchto enzymů jsou tak nadějné cíle při hledání nových forem léčby.

Klíčová slova: lymfomy, epigenetická regulace, histonové modifikace, KMT2D, EZH2, CREBBP, EP300

Abstract

Lymphomas are a diverse group of malignant tumors that arise from lymphocytes, commonly affecting lymph nodes or the spleen. They are one of the most common types of tumors worldwide. Unfortunately, many subtypes have a poor prognosis, or are not currently fully curable with standard therapeutic approaches.

Mutations in enzymes responsible for posttranslational modifications of histones are very common in certain subtypes of lymphoma, as well as in many other cancer types. These enzymes directly affect gene expression by changing the condensation state, and thus the accessibility, of chromatin. Some of these enzymes have been found to play an important role in the formation of germinal centers in lymphoid follicles. Therefore, their mutations can lead to uncontrolled proliferation and cancer development. Since many conventional therapeutic strategies are incapable of curing a large portion of lymphomas, novel and more targeted approaches are needed. Inhibition and/or modulation of the function of the aforementioned enzymes may be a basis for such approaches.

Key words: lymphomas, epigenetic regulation, histone modifications, KMT2D, EZH2, CREBBP, EP300

List of abbreviations

5mC	5-methylcytosine
ABC-DLBCL	activated B cell-like diffuse large B cell lymphoma
ARID1A	AT-rich interaction domain 1A
Ash2L	ASH2-like
BCL	B cell lymphoma
BCL2	B cell lymphoma 2
BCL6	B cell lymphoma 6
BCR	B cell receptor
BET	Bromodomain and Extraterminal domain
CARD11	Caspase Recruitment Domain Family Member 11
CCND1	cyclin D1
CD4	cluster of differentiation 4
CD40	cluster of differentiation 40
CD40L	cluster of differentiation 40 ligand
CD8	cluster of differentiation 8
CDKN1A	Cyclin Dependent Kinase Inhibitor 1A
CENP-C	Centromere Protein
Cfp1	CXXC finger protein 1
CGI	CpG island
CHD1	Chromodomain Helicase DNA Binding Protein 1
CIITA	Class II Major Histocompatibility Complex Transactivator
CREBBP	Cyclic adenosine monophosphate Response Element Binding protein Binding Protein
DNMT	DNA methyl transferase
DOT1L	Disruptor of telomeric silencing 1-like
DSB	double strand break
EBV	Epstein-Barr virus
EED	Embryonic Ectoderm Development
EP300	E1A binding protein P300
ER	estrogen receptor
ESCs	embryonic stem cells
EZH2	enhancer of Zeste 2
FDA	Food and Drug Administration
FL	follicular lymphoma

FOXA1	Forkhead Box A1
GC	germinal center
GCB-DLBCL	germinal center B cell-like diffuse large B cell lymphoma
HAT	histone acetyl transferase
HDAC	histone deacetylase
HDM	histone demethylase
HMT	histone methyl transferase
HP1	heterochromatin protein 1
IL-4	interleukin 4
IRF4	Interferon Regulatory Factor 4
JARID2	Jumonji And AT-Rich Interaction Domain Containing 2
KAT	lysine acetyl transferase
KAT6A	lysine acetyl transferase 6A
KDM	lysine demethylase
KDM6A	lysine demethylase 6A
KMT2D	lysine methyl transferase 2D
MBD	methyl-CpG-binding domain
MeCP2	methyl CpG binding protein 2
MEF2B	Myocyte Enhancer Factor 2B
MHC	major histocompatibility complex
NF-κB	Nuclear Factor Kappa B
NHEJ	non-homologous end joining
NK	natural killer
NOS	not otherwise specified
P-TEFb	positive transcription elongation factor b
PCNA	proliferating cell nuclear antigen
PRC2	Polycomb repressive complex 2
PRDM1	PR/SET Domain 1
SAM	S-adenosylmethionine
SIRT	sirtuins
SUZ12	Suppressor Of Zeste 12 protein homolog
SWI/SNF	SWItch/Sucrose Non-Fermentable
TAF3	TATA-Box Binding Protein Associated Factor 3
TE	transposable element
TET	Ten-eleven translocation
TF	transcription factor

TFIID	transcription factor II D
TLR	Toll-like receptor
TNFα	tumor necrosis factor α
Treg	regulatory T cell
UHRF1	Ubiquitin-like, containing PHD and RING finger domains 1
WHO	World Health Organization
WRAD complex	consisting of: WDR5, RbBP5, ASH2L, and DPY-30

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1. Introduction

In eukaryotes, gene expression levels are partially regulated by controlling the condensation state of chromatin on gene regulatory elements, such as promoters and activators. This can be done by epigenetic modifications – the reversible introduction of chemical marks, such as methyl or acetyl groups, to chromatin. This can take place either at the level of DNA itself, or on histone proteins. Enzymes that either make or recognize these marks can therefore serve to regulate a large number of different genes, and stand at the crossroads of entire regulatory networks.

Recently, the role of histone-modifying enzymes in various types of cancer has been extensively studied. In several different subtypes of lymphoma, mutations in these enzymes are especially frequent. And while they are not usually sufficient to cause lymphoma formation by themselves, they can serve to accelerate its growth and dissemination by working with other mutant proteins.

In this thesis, both epigenetic modifications and lymphomas as a whole are briefly introduced, with a particular focus on histone modifications and their role and frequency in lymphomas. In the sections that follow, four of the most commonly mutated histone-modifying enzymes in lymphoma are introduced. The first two, KMT2D and EZH2, are methyl transferases, while the latter, CREBBP and EP300, are acetyl transferases. Their biological and pathological functions are described, and their role in B cell development and lymphomagenesis is explored in detail, including potential cancer therapies that target these enzymes.

2. Epigenetic regulation of gene expression

Epigenetics is a subfield of genetics that is concerned with changes in phenotype that are not caused by a change in the primary DNA sequence. Epigenetic mechanisms are the reason why multicellular organisms are able to have incredibly specialized cell populations, all of which (with a notable exception in the form of lymphocytes) nevertheless contain the same DNA. This is achieved by manipulating the secondary structure of DNA – how tightly it is packaged together, and therefore how accessible it is to transcription proteins.

The primary mechanism of epigenetic control is the distribution of epigenetic marks on chromatin, which are capable of directly or indirectly changing its level of condensation. They include the addition of methyl groups onto nucleotides (especially cytosine), and various post-translational modifications of histone proteins. These marks can then recruit other chromatin-modifying proteins, such as those that change the distance between neighboring nucleotides or exchange canonical histones for non-canonical ones with different properties and functions. And although they have only become heavily researched recently, it has become clear that non-coding RNAs also play a role in the regulation of gene expression and can be considered epigenetic modifiers.

2.1. DNA methylation

By far the most common type of DNA methylation is that of cytosine at the 5th position, creating 5-methylcytosine (5mC), mainly as part of a CG dinucleotide. These CpG sites are not very common in vertebrate genomes as they are considered mutational hotspots[1] (5mC, when deaminated, converts to thymine, and is therefore not easily recognized as an unnatural base by DNA repair mechanisms[2]), but where they do occur, they are usually methylated. In this context, the exception are the so-called CpG islands (CGIs), which are about 1kbp long DNA sequences notable for their high CpG content, and for usually being unmethylated.

DNA methylation plays an essential role in the regulation of gene expression. When occurring within promoters or the first exon,[3] it functions as a transcription-silencing signal. Methylated CpG sites also serve as binding sites for proteins, which can then affect chromatin structure. In this way, DNA methylation allows for the silencing of transposable elements,[4,5] stable X chromosome inactivation,[6] and imprinting.[7]

The group of enzymes catalyzing DNA methylation, the DNA methyl transferases (DNMTs), do so by initiating a nucleophilic attack, using S-adenosyl methionine (SAM) as the source of the methyl group. There are three DNMTs in mammals – DNMT1, DNMT3a, and DNMT3b (an enzyme called DNMT2 does exist, but appears to only methylate tRNA[8,9]). Another related molecule, DNMT3L, has lost its catalytic function, but has been shown to interact with the other two DNMT3s.[10]

DNMT1 is the most active during the S phase,[11] where it processively methylates DNA following replication, which allows for the inheritance of methylation marks from mother to daughter cell. DNMT1 shows a greater affinity for hemimethylated DNA by itself,[12] but *in vivo*, its processivity is further increased by its association with the replication machinery.[13] PCNA (proliferating cell nuclear antigen – a "sliding clamp" protein that increases the processivity of DNA polymerase δ) has been shown to bind to DNMT1, specifically recruiting it to hemimethylated sites that have just been replicated, and therefore increasing the efficiency of methylation.[14]

Unlike DNMT1, DNMT3a and DNMT3b do not show any preference for hemimethylated DNA – rather, they help establish methylated sequences (the so-called *de novo* methylation). They are especially active during the embryonic stage, where they are required for successful development,[15] as well as during gametogenesis.[7] In adults, DNMT3 levels are quite low in most tissues – they are, however, frequently elevated in cancer cells.[16] DNMT3L is a structurally related molecule that lacks the catalytic function of other DNMTs. It cannot bind to DNA itself[17] – but it does bind both to the other DNMT3 proteins[18] and to unmethylated lysine on histone 3 (H3K4).[19] DNMT3L likely increases the affinity of DNMT3a for those areas,

and generally functions as a co-regulator. The importance of DNMT3L can be illustrated by the fact that it has been shown to be indispensable during imprinting and the silencing of retrotransposons.[20,21]

DNA demethylation also serves an important function in regulating gene expression, and often accompanies histone modifications that lead to a greater transcriptional accessibility of DNA. The TET (Ten-eleven translocation) family of demethylating enzymes first oxidizes 5mC in a series of steps[22] – the resulting base is then recognized as abnormal and exchanged for an unmethylated cytosine in a process very similar to base excision repair.[23] Demethylation is especially important during early embryonic development – a large wave of demethylation occurs in the early embryo, which erases a significant part of the parents' methylation patterns, and is followed by *de novo* methylation by DNMT3s.

Both DNMTs and TET enzymes are involved in interactions with various other proteins. For instance, apart from PCNA, DNMT1 also forms a complex with UHRF1 (Ubiquitin-like, containing PHD and RING finger domains 1), which can bind to hemimethylated DNA,[24] and whose knock-down leads to a decrease in the association of DNMT1 with chromatin and to decreased methylation rates.[25] The loss of either DNMT1 or UHFR1 is pro-inflammatory, likely due to hypomethylation and thus over-expression of transposable elements (TEs), which activates the same Tumor Necrosis Factor α (TNFα)-dependent pathway as viral nucleic acids.[5] Some proteins can direct enzymes to specific parts of the genome – such is the case with DNTM3b, which interacts with CENP-C (Centromere protein C), with the knock-down of either one of them leading to disruption of chromosomal segregation during mitosis.[26]

DNMTs can also associate with transcription factors – for example, DNMT3a can bind to the tumor suppressor p53 and suppress its activation of the transcription of p21.[27] Another infamous transcription factor, MYC (which is frequently mutated in Burkitt's lymphoma[28]), recruits DNMT3a to the *CDKN1A (Cyclin Dependent Kinase Inhibitor 1A)* promoter, where it acts as a co-repressor.[29] *CDKN1A* codes for the TF p21, which is normally expressed following DNA damage. Therefore, these interactions are relevant for genome stability and related tumorigenesis.

The dysfunction of either DNA methylating or demethylating enzymes can lead to a number of diseases. Of special interest is epigenetic deregulation in cancer. In tumor cells, the entire landscape of DNA methylation can change, with genome-wide hypomethylation, and hypermethylation of CpG islands being especially common. As mentioned previously, hypomethylation can re-activate transposons, which can cause mutations and lead to tumorigenesis.[30] A change in the methylation status of certain genes can lower the expression of tumor-suppressor genes, such as those responsible for cell cycle arrest or DNA damage repair,[31] or increase the expression of oncogenes, such as those responsible for proliferation.[32]

2.2. Histone modifications

In eukaryotes, DNA is periodically wrapped around globular protein structures called nucleosomes. Each nucleosome is made up of eight histone proteins – a tetramer of the H2A-H2B proteins, and two H3-H4 dimers. Another histone protein, H1, binds to and regulates linker DNA, which connects neighboring nucleosomes. Histones are strongly conserved basic proteins that allow for the efficient packaging of DNA inside the nucleus. Their basicity lends them a strong positive charge, which allows them to interact with the negatively charged DNA.

Each histone can be extensively post-translationally modified, with modifications of their N-termini being especially common. Some of those modifications include methylation, acetylation (or other acylations), phosphorylation, ubiquitination or SUMOylation.

Histone methylation occurs at either lysine or arginine residues and each residue can be either mono-, di-, or trimethylated, with different degrees of methylation having different effects. Histone methylation can have either an activating or a repressive effect on transcription, depending on what proteins are recruited by each specific mark. These proteins can then interact with other histone-modifying enzymes, DNMTs, and chromatin-remodeling enzymes.

One of the most common types of methylation is that of lysine 4 on histone 3 (H3K4me). In humans, six H3K4 methyl transferases have been identified, and they all make up the KMT2 (lysine methyl transferase 2) family. All six are capable of monomethylation, but vary in their ability to di- and trimethylate H3K4.[33] H3K4 methylation is usually associated with active chromatin, although different degrees of methylation are enriched in different regions – for example, the KMT2A complex, which is capable of trimethylation, contains the Cfp1 (CXXC finger protein 1) subunit, which binds unmethylated CpG sites at promoters of actively transcribed genes.[34] This leads to H3K4me3 being enriched at promoters, where the TAF3 (TATA-Box Binding Protein Associated Factor 3) subunit of the transcription factor TFIID can bind to it directly, which makes the formation of a stable pre-initiation complex more likely.[35] The strength of this binding is further increased by the presence of an acetylated lysine 9 on histone 3 (H3K9ac)[35] – which is functionally consistent, since H3K9ac is also associated with active transcription. Another member of the KMT2 family, KMT2D, which will be discussed in detail later, is primarily a monomethyltransferase, and is especially enriched at active enhancers, where H3K4me1/2 allows for the binding of transcription factors.[36] Apart from binding effector proteins, H3K4me also prevents the binding of DNMTs to histones.[19]

Other histone methylations, such as that of histone 3 on lysine 9 (H3K9) or 27 (H3K27), have a repressive effect. H3K27me marks are made by the Polycomb repressive complex 2 (PRC2), which seems to have a preference for CpG islands without activating motifs.[37] It also exhibits both "writer" and "reader" activities – its EED (Embryonic Ectoderm Development) subunit binds to H3K27me3, which enhances the methyl transferase activity of the catalytic subunit, EZH2, thus allowing for the spread of the repressive mark throughout the promoter.[38] H3K27me3 commonly occurs together with H3K4me3 at so-called "bivalent promoters" with high CpG content in embryonic stem cells (ESCs). These modifications have opposing functions, and so these promoters exhibit a low level of transcription – but during differentiation, either of these marks can get removed, which determines cell fate.[39] The PRC2 complex also seems to recruit Tet1, which keeps these bivalent promoters in a hydroxymethylated state.[40] A H3K27 demethylase, KDM6A, serves as an example of the dynamic relationships between histone modifications – when it is expressed, it not only removes repressive methylation marks from H3K27, but also recruits KMT2D, which methylates H3K4 and allows for active transcription.[41]

Repressive H3K9 methylation is also common. It is especially associated with DNMTs, with UHRF1 being able to bind H3K9me3 and recruit DNMT1 to sites marked by it. Remarkably, UHRF1 also has its own histone-modifying activity. Once recruited by H3K9me3, it catalyzes monoubiquitination at lysine 18 or 23 on histone 3 – these modifications are then recognized by DNMT1 and activate it.[42] The most prominent H3K9 methyl transferases, SUV39H1/2 and G9a, also seem to interact with DNMT1 directly.[43]

A complicated interplay exists between DNA methylation and histone modifications. This interaction goes both ways – DNA (de)methylases are more likely bind to specific modifications (e.g. the affinity of DNMT3s for unmethylated H3K4,[19] or that of DNMT1 for monoubiquitinated H3K18[44]), and their interaction with chromatin can recruit histone-modifying enzymes to specific sites. This recruitment is often indirect – methylated CpG sites can be recognized by proteins containing a methyl-CpG-binding domain (MBD), which then recruit other enzymes that modify chromatin. One example of this could be the protein MeCP2 (methyl CpG binding protein 2), which binds to CpG sites with its MBD domain and recruits histone deacetylases, which make chromatin less transcriptionally active.[45]

Histone acetylation generally activates transcription through a well-understood mechanism. While a lysine residue is unacetylated, its positive charge allows it to interact closely with the negatively-charged DNA. But upon acetylation, this positive charge is lost and DNA becomes more loosely attached to histones and thus more accessible to TFs. Acetylation marks can also be recognized by proteins containing the bromodomain, such as the BET (Bromodomain and Extraterminal domain) protein family, members of which can promote transcription by interacting with transcription elongation factors, such as P-TEFb (Positive Transcription Elongation Factor b).[46]

Some of the most well-known histone acetyl transferases (HATs) are the enzymes CREBBP and EP300, which can acetylate histones at many different positions.[47] These proteins often form a complex together, although they do have separate functions as well. Apart from regulating gene expression, they have been shown to play a role in the double strand break (DSB) response. CREBBP/EP300-mediated acetylation of the RAD52 protein is necessary for its prolonged accumulation at DSB sites and the consequent strand invasion during DNA repair by homologous recombination.[48] Histone deacetylases (HDACs) from the sirtuin (SIRT) family are also involved in DSB repair. SIRT2/3 mediate RAD52 deacetylation[48] and SIRT6 serves as a DSB sensor.[49] CREBBP/EP300 are also involved in DSB repair by non-homologous end joining (NHEJ). Here, they acetylate histones near DSB sites, which helps recruit proteins involved in NHEJ.[50]

Bromodomains, which recognize acetylated lysine, have already been mentioned. Another domain, called the chromodomain, recognizes methylated lysine, and both of these domains allow for the recruitment of proteins to epigenetic marks. Bromodomain and/or chromodomain-containing proteins include chromatin-remodeling complexes, such as the ATP-dependent CHD1 (Chromodomain Helicase DNA Binding Protein 1). CHD1 binds selectively to H3K4me with its two chromodomains.[51] CHD1 is likely involved in the reorganization of nucleosomes during transcription by RNA polymerase II, since its loss leads to shorter, incomplete transcripts.[52] On the repressive side, the HP1 (Heterochromatin Protein 1) protein binds to the H3K9me mark and promotes chromatin condensation.[53] It is also involved in the maintenance of epigenetic marks following replication.[54] These complexes also play a role during various forms of DNA repair. During NHEJ, one of the proteins recruited by CREBBP/EP300 is the complex SWI/SNF (SWItch/Sucrose Non-Fermentable), which rearranges the position of nucleosomes at DSB sites, making the accumulation of proteins involved in the repair easier.[50]

The complex role of histone modifications (and epigenetics in general) in cancer is extensive and only just beginning to be fully explored. Broadly speaking, the dysfunction of any of the aforementioned proteins can lead to tumorigenesis. The exact effect may depend on concurrent mutations of other genes, or on the specific cell type they occur in. The aforementioned H3K27me3 demethylase KDM6A has been shown to be elevated in a breast cancer cell line, with its knockdown leading to decreased proliferation and invasiveness – but no such decrease took place when it was knocked down in osteosarcoma cells.[41] To complicate matters further, in some cancers, the effects of histone-modifying enzymes may be structural rather than enzymatic.[55] In those cases, inhibiting the catalytic function of said enzyme would not cause sufficient decrease in proliferation. That is not to say that such inhibition has not seen successfully tested. For example, the inhibition of EZH2 has shown potential in reducing proliferation of cancer cells with mutated ARID1A (AT-rich Interaction Domain 1A), which is a component of the SWI/SNF complex.[56]

3. Lymphomas

Lymphomas are a diverse group of malignant tumors characterized by the proliferation of lymphocytes – B cells, T cells, or NK cells. They typically arise in the lymph nodes, although they can also develop in other sites of the lymphatic system, such as the spleen or bone marrow. Lymphomas that arise from B cells are the most common, comprising about 90% of all lymphomas, while T/NK cell-derived lymphomas are much rarer. During B cell activation, somatic hypermutation and class-switch recombination take place – these events introduce point mutations to the V-regions of antibody genes and cause the antibody to switch to a different isotype, respectively. The enzyme involved in both of these processes is the activation-induced cytidine deaminase (AID). Importantly, it can sometimes cause mutations in non-immunoglobulin DNA regions as well. This is the most commonly accepted reason for the difference in prevalence of B compared to T/NK lymphomas.[57]

Lymphomas have traditionally been divided into Hodgkin and non-Hodgkin lymphomas. Hodgkin lymphoma is now classified as a specific subtype of mature B-cell lymphomas, with distinct histology (such as the presence of Reed-Sternberg cells) and a generally good prognosis.[58] Nowadays, lymphomas are divided into B cell and T/NK cell lymphomas and into further subcategories based on cell-of-origin classification.

According to the 5th edition of the World Health Organization (WHO) classification of hematolymphoid tumors (published in 2022), there are four main categories of B cell lymphoid malignancies: tumor-like lesions with B cell predominance, precursor B cell neoplasms, mature B cell neoplasms, and plasma cell neoplasms and other diseases with paraproteins. Given the scope of this thesis, the most important are mature B cell neoplasms (summarized in Table 1). Apart from cell-of-origin classification, this classification also utilizes specific types of tumor-associated genetic changes (e.g. diffuse large B cell lymphoma with *MYC* and *BCL2* rearrangements).

Table 1. WHO classification of hematolymphoid tumors – mature B cell neoplasms [59]

Pre-neoplastic and neoplastic small lymphocytic proliferations • Monoclonal B-cell lymphocytosis • Chronic lymphocytic leukemia/small lymphocytic lymphoma
Splenic B-cell lymphomas and leukemias • Hairy cell leukemia • Splenic marginal zone lymphoma • Splenic diffuse red pulp small B-cell lymphoma • Splenic B-cell lymphoma/leukemia with prominent nucleoli
Lymphoplasmacytic lymphoma • Lymphoplasmacytic lymphoma
Marginal zone lymphoma • Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue

- Primary cutaneous marginal zone lymphoma
- Nodal marginal zone lymphoma
- Pediatric marginal zone lymphoma

Follicular lymphoma
- In situ follicular B-cell neoplasm
- Follicular lymphoma
- Pediatric-type follicular lymphoma
- Duodenal-type follicular lymphoma

Cutaneous follicle center lymphoma
- Primary cutaneous follicle center lymphoma

Mantle cell lymphoma
- In situ mantle cell lymphoma
- Mantle cell lymphoma
- Leukemic non-nodal mantle cell lymphoma

Transformations of indolent B-cell lymphomas
- Transformations of indolent B-cell lymphomas

Large B-cell lymphoma
- Diffuse large B-cell lymphoma, NOS
- Diffuse large B-cell lymphoma/ high grade B-cell lymphoma with *MYC* and *BCL2* rearrangements
- ALK-positive large B-cell lymphoma
- Large B-cell lymphoma with *IRF4* rearrangement
- High-grade B-cell lymphoma with 11q aberrations
- Lymphomatoid granulomatosis
- EBV-positive diffuse large B-cell lymphoma
- Diffuse large B-cell lymphoma associated with chronic inflammation
- Fibrin-associated large B-cell lymphoma
- Fluid overload-associated large B-cell lymphoma
- Plasmablastic lymphoma
- Primary large B-cell lymphoma of immune-privileged sites
- Primary cutaneous diffuse large B-cell lymphoma, leg type
- Intravascular large B-cell lymphoma
- Primary mediastinal large B-cell lymphoma
- Mediastinal gray zone lymphoma
- High-grade B-cell lymphoma, NOS

Burkitt lymphoma
- Burkitt lymphoma

HSHV/HHV8-associated B-cell lymphoid proliferations and lymphomas
- Primary effusion lymphoma
- KSHV/HHV8-positive large B-cell lymphoma
- KSHV/HHV8-positive germinotropic lymphoproliferative disorder

Lymphoid proliferations and lymphomas associated with immune deficiency and dysregulation
- Hyperplasias arising in immune deficiency/dysregulation
- Polymorphic lymphoproliferative disorders arising in immune deficiency/dysregulation
- EBV-positive mucocutaneous ulcer
- Lymphomas arising in immune deficiency/dysregulation
- Inborn error of immunity-associated lymphoid proliferations and lymphomas

Hodgkin lymphoma
- Classic Hodgkin lymphoma
- Nodular lymphocyte predominant Hodgkin lymphoma

Among B cell lymphomas, the most common type by far is diffuse large B cell lymphoma (DLBCL), which accounts for approximately 40% of all non-Hodgkin lymphoma cases. DLBCL could be divided into two broad subcategories – germinal center B cell-like DLBCL (GCB-DLBCL) and activated B cell-like DLBCL (ABC-DLBCL, also called non-GCB). These subcategories were defined based on the similarity of their tumor cells to normal B cells. Both types are related to the germinal center B cells of secondary lymphoid organs (Figure 1). GCB-DLBCL-type cells arise from GC B cells (also called centroblasts) normally occurring in the dark zone of lymphoid follicles, which undergo somatic hypermutation and clonal expansion. ABC-DLBCL-type cells arise primarily from GC B cells (also called centrocytes) in the light zone of lymphoid follicles, which is where the process of clonal selection normally takes place. This precise DLBCL sub-classification is biologically and clinically important, as these two subtypes differ in their genetic background as well as prognosis, which is worse in ABC-DLBCL in comparison to GCB-DLBCL.[60]

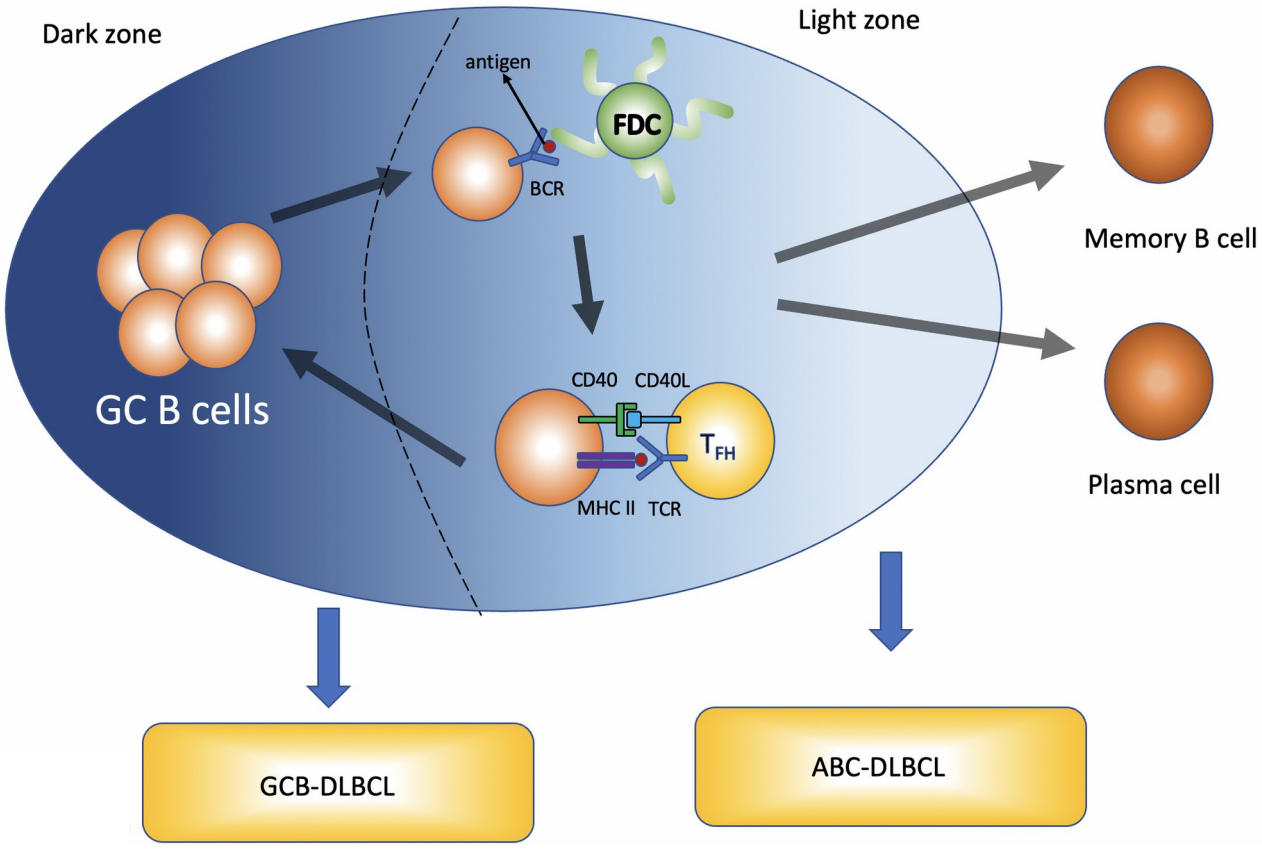

Figure 1. The germinal center reaction and its relation to DLBCL subtypes

ABC-DLBCL – activated B-cell-like diffuse large B cell lymphoma; BCR – B-cell receptor; CD40 – cluster of differentiation; CD40L – cluster of differentiation 40 ligand; FDC – follicular dendritic cell; GCB-DLBCL – germinal center B-cell-like diffuse large B cell lymphoma; MHC II – major histocompatibility complex II; TCR – T-cell receptor; T_{FH} – follicular helper T cell

The division of lymphomas into aggressive and indolent types is also of clinical significance. Aggressive lymphomas are characterized by rapid proliferation and disease progression, while indolent lymphomas progress more slowly. In fact, after a diagnosis of follicular lymphoma (FL), which is a typical indolent lymphoma, a "watch and wait" approach can even be taken.[61] However, follicular lymphomas still gradually progress, and can transform into DLBCL, which is associated with much lower survival rates.[62] It is also important to note that more aggressive lymphomas do not always convey a worse prognosis, at least as long as they are diagnosed early and appropriate therapy is administered. For example, Burkitt lymphoma, which is a rare form of aggressive lymphoma predominantly diagnosed in children, is highly treatable with chemotherapy.[63]

It has been shown that different types of BCL are associated with different sets of mutations. Epigenetic dysfunction seems to be much more common in more indolent types of lymphoma, such as FL[64], and in GCB-DLBCL.[65] About 50% of GCB-DLBCL cases have been found to possess a mutation in a chromatin-modifying enzyme,[66] while as much as 96% of FL cases have at least one such mutation.[67] The most common aberrations of epigenetic regulators include inactivating mutations of KMT2D and CREBBP, and activating mutations of EZH2.[66] For example, CREBBP and EP300 mutations are very common in both GCB-DLBCL and FL.[68] The inactivating mutations of either lead to decreased levels of antigen presentation (which serves as an immune evasion strategy), and in the case of CREBBP, to germinal center B cell hyperplasia. The tumor suppressor p53 is also a normal target of acetylation by CREBBP, and defective CREBBP can interfere with its ability to facilitate the DNA damage response.[69] EZH2, on the other hand, is commonly affected by gain-of-function mutations in lymphoma, leading to increased silencing of its target genes. This prevents GC B cells from differentiating into plasma cells or memory B cells, and keeps them "trapped" in the GC B phenotype.[70] Apart from histone-modifying enzymes, follicular lymphoma in particular has a high incidence of mutations in linker histone proteins, which can sometimes stop them from binding DNMTs.[71]

In ABC-DLBCL, epigenetic dysregulation is not as common as in the GCB subtype. These tumors are frequently associated with mutations leading to constitutively active NF-κB (Nuclear Factor Kappa B) signaling. Those are, for example, mutations in their B cell receptor (BCR) that cause it to be permanently active,[72] or mutations anywhere downstream of the receptor, such as in the adaptor protein CARD11 (Caspase Recruitment Domain-containing protein 11).[73]

Other non-epigenetic mutations are also common in many types of lymphoma. The BCL2 (B Cell Lymphoma 2) family of proteins is associated with lymphomas in general. For example, BCL6 is a transcriptional repressor that is required for germinal center formation[74], and that is frequently constitutively active in DLBCL. Since BCL6 downregulation is required for B cells to leave the germinal center and mature into plasma cells,[75] activating mutations can lead to a stop in B cell differentiation, one of the

hallmarks of cancer. BCL6 target genes may also undergo translocations, which helps them avoid BCL6-mediated repression.[76] For example, *MYC* translocation, which is a defining feature of Burkitt lymphoma,[77] leads to the loss of BCL6-mediated repression of this proto-oncogene that normally takes place in the dark zone of germinal centers.[78] Other common events in lymphoma are the downregulation of MHC class I molecules, which help cancer cells avoid immune recognition and immune system-mediated elimination,[79] and mutations that lead to the overall expansion of germinal centers.[80]

It is important to note that there is not necessarily a clear line between epigenetic and non-epigenetic dysregulation – for example, a mutation in BCL6 is not a direct mutation in a histone-modifying enzyme, but since BCL6 represses its target genes at least partially by recruiting histone deacetylases,[81] the effects of it can be considered epigenetic. Epigenetic modifiers and other proteins affect each other, and can either exacerbate or mitigate the tumorigenic effects of the other.

4. Histone-modifying enzymes in lymphoma

A variety of enzymes that play a role in epigenetic regulation have been associated with lymphoma, as well as with other types of cancer. The following four – KMT2D, EZH2, CREBBP, and EP300 – are by far the most frequently mutated in lymphoma, particularly in GCB-DLBCL and FL. Some other frequently mutated proteins in lymphoma include other histone-modifying enzymes (such as KMT2C), chromatin-remodeling proteins, DNA methyl transferases, and histone proteins.

All four of these enzymes are important for either the development of germinal centers, or for the exit of cells from germinal centers. Nevertheless, it appears that the mutation of any single one of these enzymes is not sufficient to induce lymphoma. Tumor cells almost never possess just a single mutation, however, and it has been clearly demonstrated that mutations in these enzymes belong to critical events in lymphomagenesis and accelerate the formation, growth, and aggressiveness of lymphomas.

Table 2 shows the approximate frequency of mutations of *KMT2D*, *EZH2*, *CREBBP*, and *EP300* in lymphoma. The epigenetic modifications made by these four enzymes are highlighted in Figure 2. One tumor or cell line can, and often does, have mutations in more than one of these genes at the same time.

Table 2. Frequency of mutations of genes coding histone-modifying enzymes in GCB-DLBCL and FL

	Frequency of mutations [%]		Sources
	GCB-DLBCL	FL	
KMT2D	30-45%	40-90%	[64,66,82,83]
EZH2	20-25%	10-30%	[84–89]
CREBBP	30-40%	~65%	[67,68,88–90]
EP300	~10%	~10%	[67,68]

CREBBP – cyclic adenosine monophosphate response element binding protein binding protein; *EP300* – E1A binding protein P300; *EZH2* – enhancer of zeste homolog 2; GCB-DLBCL – germinal center B-cell-like diffuse large B cell lymphoma; FL – follicular lymphoma; *KMT2D* – lysine methyltransferase 2D

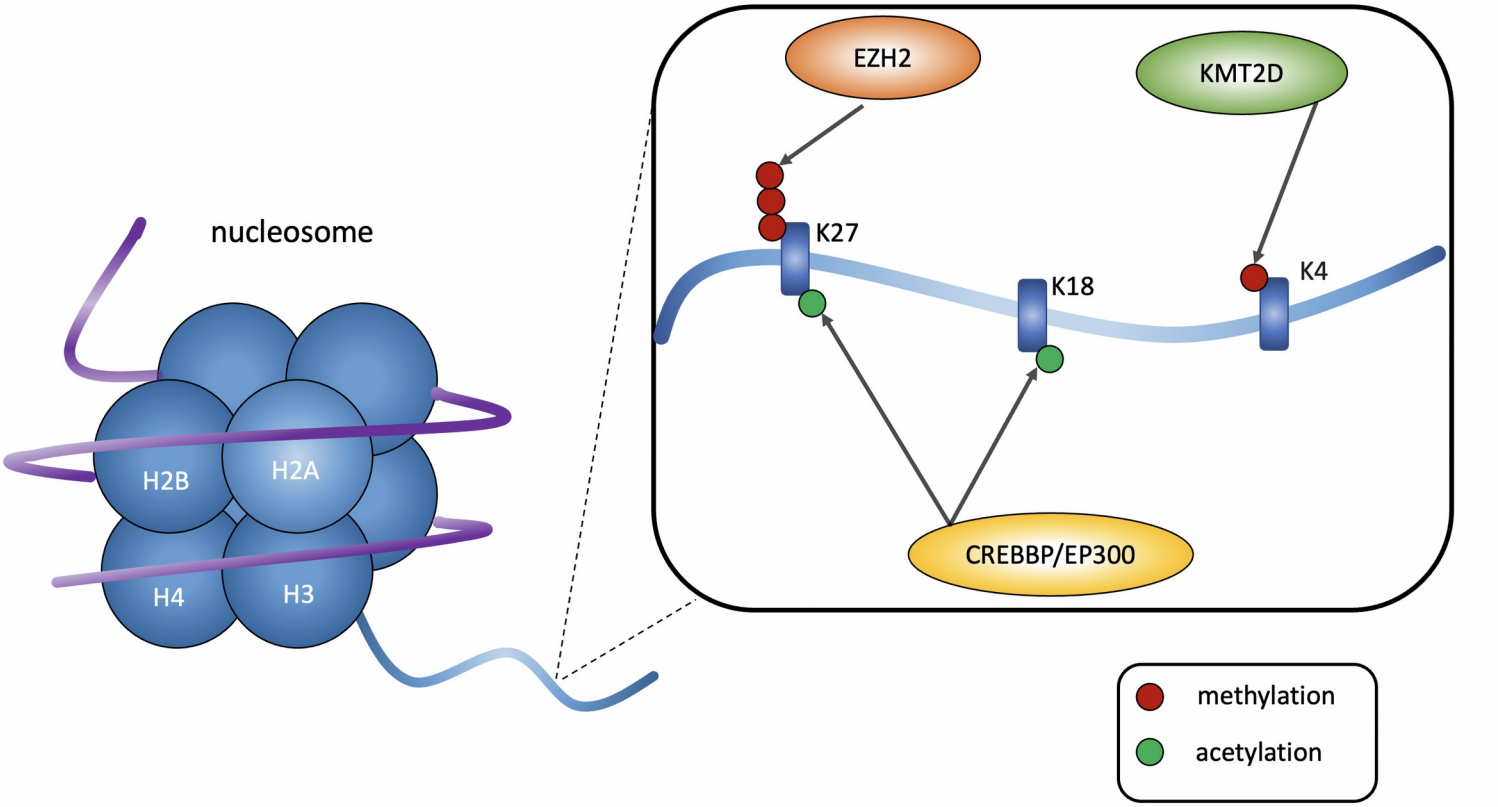

Figure 2. Epigenetic modifications made by KMT2D, EZH2, and CREBBP/EP300

CREBBP – cyclic adenosine monophosphate response element binding protein binding protein; *EP300* – E1A binding protein P300; *EZH2* – enhancer of zeste homolog 2; *KMT2D* – lysine methyltransferase 2D
K4 – histone 3, lysine 4; K18 – histone 3, lysine 18; K27 – histone 3, lysine 27

4.1. KMT2D

4.1.1. Structure and function

A member of the KMT2 family of histone methyl transferases (HMTs), KMT2D is primarily responsible for the monomethylation of lysine 4 on histone 3 (H3K4me1), although it has some di- and trimethylase activity as well. H3K4me1 marks are present ubiquitously on enhancers, while H3K4me2/3 are more commonly found on promoters. In embryonic stem cells (ESCs), H3K4me1 appears at poised enhancers, together with the H3K27me3 mark, and at active enhancers, together with the H3K27ac mark.[91] KMT2D has been shown to directly recruit the acetyl transferases CREBBP and EP300, which then deposit the H3K27ac mark to activate enhancers.[92] Chromatin with the latter combination of histone modifications preferentially associates with RNA polymerase II, showing the importance of KMT2D in directing cell fate.[91]

Under normal physiological conditions, KMT2D is necessary for a number of biological processes. These include the formation of heart muscle[93] and brown adipose tissue,[92] immunoglobulin class switching[94] and, most importantly to lymphomagenesis, germinal center development.[82] With this many roles, it is not surprising that a whole-body knockout leads to embryonic lethality.[36] Germline loss-of-function mutations lead to the rare Kabuki syndrome, which is characterized by various developmental abnormalities.[95]

KMT2D (and the closely related KMT2C) associates with other proteins to form a multi-subunit complex, where it functions as a scaffold protein, without which the entire complex becomes unstable.[96] The KMT2D protein complex includes the sub-complex WRAD, which has been shown to bind to several transcription factors and likely recruits the entire complex to specific genomic loci.[97] Another component of the KMT2D complex is KDM6A, a lysine demethylase which removes methyl groups from H3K27. Trimethylation on H3K27 is a repressive mark made by EZH2 that occurs together with H3K4me3 at bivalent promoters in embryonic stem cells. When the KMT2D complex is recruited to a specific promoter, the KDM6A protein removes the H3K27me3 marks. This allows for the acetylation of H3K27 by CREBBP/EP300 and activates transcription from said promoter, an important step during differentiation.[98] During this process, KMT2D also seems to trimethylate these promoters, and has been shown to be indispensable for Hox gene methylation.[99]

4.1.2. Role in lymphomagenesis

The association of KMT2D with lymphomas has been clearly demonstrated. KMT2D mutations have been shown to be associated with lower survival rates in different types of lymphoma.[100,101] Although statistics differ, the rate of mutations seems to be about 40% for GCB-DLBCL cases, and as much as 90% for FL cases.[64,90] KMT2D mutations are loss-of-function and they prevent the methylation (and thus activation) of tumor-suppressor genes.[66] The most common type are nonsense mutations that introduce a premature stop codon, which leads to a truncated protein product usually lacking the entire catalytic SET domain at the C terminus.[82]

KMT2D is likely necessary for the formation of normal germinal centers. It puts many enhancers into a poised state, in which transcription is repressed, but can be activated by later modifications. The enhancers concerned are ones that promote cell cycle arrest and terminal differentiation. This is essential in germinal centers, since GC B cells need to proliferate rapidly and only differentiate once they have undergone clonal selection. During lymphomagenesis, KMT2D mutations typically occur early on, before the initiation of the GC reaction. Such a loss leads to an increase in the number of GC B cells in secondary lymphoid organs and an overall expansion of the GC compartment.[82] Upon immunization, KMT2D-deficient mice also retain large GCs for longer.

Nevertheless, the mutation of KMT2D alone is not likely to lead to the development of lymphomas with the usual markers of GC-derived cells. During the development of typical GC-derived tumors, such as follicular lymphoma, KMT2D loss usually serves to drive the progression of tumors which already have an upregulated BCL2 expression. BCL2 is an anti-apoptotic protein, frequently altered in many different types of lymphoma. Compared to those with BCL2 alterations only, tumors with an added KMT2D deficiency grow larger, have bigger numbers of tumor cells, and are more likely to invade surrounding tissues.[83] KMT2D deficiency itself also leads to the upregulation of BCL2, pointing to a positive feedback loop.[82]

KMT2D has been shown to target numerous genes, many of which are involved in signaling pathways important for B cell development and migration. A direct correlation has been shown between KMT2D levels, the abundance of H3K4me1 marks on enhancers, and the expression levels of genes whose products are involved in the CD40 or NF-κB signaling pathways. Since H3K4me1 marks usually activate gene expression, KMT2D loss leads to a downregulation of its target genes in most cases.[83]

KMT2D loss also seems to make cells more resistant to apoptosis – for instance, while CD40 signaling triggers proliferation in normal B cells, it often leads to apoptosis in DLBCL cells.[102] Those cells that are KMT2D-deficient, however, were found to be more resistant to CD40-induced apoptosis, likely because some components of the signaling pathway are targets of KMT2D.[83]

More broadly, KMT2D (and other KMT2 family members) has been shown to interact with the tumor suppressor p53. After the induction of DNA damage, KMT2D associates with p53, and allows for the expression of its target genes. When KMT2D is knocked down, the levels of p53-induced proteins, such as p21, decrease. KMT2D knock down cells also have a higher level of the γH2AX histone variant, which is a marker of DNA damage.[103] Ash2L (ASH2-Like), which is a member of the protein complex common to all KMT2 enzymes, has been shown to specifically regulate the p53-dependent expression of pro-apoptotic genes.[104] However, this type of Ash2L-mediated regulation occurs on promoters rather than enhancers, which means that other members of the KMT2 family likely play a bigger role here than KMT2D does. The p53 protein itself could also be subject to methylation – this has not been shown with KMT2D yet, but has been proven with KMT2B, suggesting that a role for KMT2 enzymes in non-epigenetic regulation is at least possible.[105]

Apart from lymphoma, KMT2D has also been connected with many other cancers, such as medulloblastoma or ovarian cancer.[106,107] In some cancer cell lines and mouse models, however, KMT2D has been shown to promote tumorigenesis, rather than acting as a tumor-suppressor. For example, in estrogen receptor-positive (ER-positive) breast cancer cells, H3K4me1 modifications allow for the binding of transcription factors, such as FOXA1 (Forkhead Box A1), which in turn facilitate the interaction of the estrogen receptor with chromatin. In these cells, KMT2D knockdown actually leads to a decrease in cancer cell proliferation.[108] The cancer-promoting role of KMT2D has also been demonstrated in leukemia cells.[109] These examples show that functional context is absolutely critical. The exact effects of KMT2D deregulation may depend on numerous factors, such as which TFs recruit it to DNA in a given cell type or what mutations are occurring together with KMT2D in a given cell population. For example, in some breast and pancreatic cancer cell lines, KMT2D (and other histone-modifying enzymes, such as KMT2A and KAT6A[110]), has been shown to bind to a mutant form of p53. This specific mutant does not act as a tumor-suppressor, but instead has newly gained pro-oncogenic functions. Thus, the knockdown of KMT2D can stop this mutant form of p53 from facilitating tumorigenesis.[111]

4.1.3. Therapeutic implications

While the U.S. Food and Drug Administration (FDA) has approved several drugs specifically targeted at EZH2 and CREBBP/EP300, the development of treatment strategies for KMT2D-mutant tumors is still in progress.

Since KMT2D mutations in lymphoma are usually loss-of-function, developing a successful treatment cannot be as simple as inhibiting the enzyme (as is the case with EZH2). Potential therapies might focus on inhibiting other epigenetic regulators that normally act in opposition to KMT2D. One way to

do so is by inhibiting specific lysine demethylases (KDMs) that share histone residue targets with KMT2D. Several inhibitors of members of the KDM5 family have been developed. Some of them were able to cause a decrease in proliferation and increase in apoptosis in both GCB-DLBCL and FL cells. Importantly, this effect was greater in KMT2D-mutant cells. Specifically, KDM5 inhibitor treatment caused the upregulation of the tumor suppressor and cell cycle regulator p21 and several negative regulators of BCR signaling. [112]

Since it is the presence of H3K4me1 marks (made by KMT2D), together with H3K427me3 marks (made by EZH2) that puts enhancers into a bivalent state, patients with KMT2D-mutant tumors could potentially benefit from EZH2 inhibition as well. Decreasing the level of H3K27me3 in these cells could restore the balance between activating and repressive marks and maintain this state of bivalency. This has been demonstrated with the KMT2C enzyme, which is closely related to KMT2D.[113]

4.2. EZH2

4.2.1. Structure and function

EZH2 is a part of the PRC2 complex formed by Polycomb group proteins. In mammals, the PRC2 complex methylates histone 3 on lysine 27 (H3K27) on all three levels (mono-, di- and trimethylation). H3K27 methylation is associated with the repression of transcription.[114] As mentioned previously, H3K27me3 modifications often occur together with H3K4me3 on bivalent promoters,[39] and with H3K4me1 on poised enhancers in embryonic stem cells.[91] The presence of poised enhancers is essential for many processes during embryonic development, such as the imprinting of certain genes,[115] as well as for normal cellular function in differentiated cells.

EZH2 is the catalytic subunit of the PRC2 complex. This complex also contains the proteins EED and SUZ12 (Suppressor Of Zeste 12 protein homolog), which are required for the catalytic activity of EZH2.[38,116] The EED subunit also has a "reader" function – it can bind to already methylated H3K27, and activate methylation by EZH2 on a neighboring nucleosome.[117] In consequence, it likely allows for the spread of this repressive chromatin mark. On the other hand, the presence of activating marks, such as H3K4me3 or H3K36me2/3 on the same histone tail, inhibits PRC2.[118] The other essential component of the complex, SUZ12, mediates interaction with some accessory proteins, such as JARID2 (Jumonji And AT-Rich Interaction Domain Containing 2).[119] In the absence of prior H3K27me marks, the methylated form of JARID2 can be recognized by the EED subunit and also activate the PRC2 complex.[120]

4.2.2. Role in lymphomagenesis

EZH2 mutations in lymphoma (as well as other types of cancer[121]) are typically gain-of-function. One specific site subject to frequent heterozygous mutations is Y641 (tyrosine 641). Mutations of Y641 are present in about 22% of GCB-DLBCL and 7% of FL cases, respectively.[86] Mutated EZH2 has an increased affinity for dimethylated H3K27 (H3K27me2), and thus increases the levels of H3K27me3 in tumor cells.[122]

A sharp physiological increase in EZH2 expression is seen during normal germinal center development. EZH2 is required for GC formation and for the maintenance of the GC B cell phenotype until cells are ready to exit the GC reaction.[123] Therefore, complete inactivation of EZH2 in GC B cells can lead to lower antibody levels and an impaired formation of immunological memory.[124] Gain-of-function mutations, however, silence genes that allow cells to eventually differentiate – such as *PRDM1 (PR/SET Domain 1)*, whose product regulates differentiation into plasma cells.[70]

This EZH2-mediated silencing in GCs is due to the creation of new bivalent promoters, which are partially different from the ones that EZH2 helps maintain during embryonic development. The new state of bivalency is something that is established in normal GC B cells too – it allows cells to transiently repress the transcription of specific genes, undergo the GC reaction, and avoid apoptosis caused by DNA damage.[124] Mutated forms of EZH2, however, tip the balance in favor of stronger continual repression, not allowing GC B cells to differentiate. The H3K27me3 mark also spreads from genes that are normally repressed in wild-type cells to new nearby promoters.[125] When treated with a specific EZH2 inhibitor, these cells begin to express genes typical for post-GC differentiation and start to resemble ABC-DLBCL cells.[70] This shows that it is the enzymatic activity of EZH2, rather than any structural role, that is primarily responsible for these changes. That is not to say that EZH2 does not also have a non-enzymatic role in tumorigenesis – for example, in NK/T cell lymphoma, it was found that increased levels of EZH2 lead to proliferation, even when its catalytic SET domain is deleted. This is because in this setting, EZH2 acts as a direct transcription factor that binds to and activates the promoter of *CCND1*, whose product, cyclin D1, in turn promotes cell cycle progression.[126]

PRDM1 was previously mentioned as a gene that is repressed by mutant EZH2 in GCs. There are many other EZH2 targets in both healthy and mutant cells, however. These include *IRF4 (Interferon Regulatory Factor 4)*, another regulator of terminal B cell differentiation, and *CDKN1A*, which encodes the tumor suppressor p21.[124] p21 inhibits cyclin/cyclin-dependent kinase complexes, and therefore functions in cell-cycle checkpoints when expressed. Active EZH2 represses p21 expression, promoting the rapid proliferation of cells in the GC compartment. Other key EZH2 targets likely include genes involved in the CD40 signaling pathway. While healthy GC B cells require a signal through this pathway from follicular helper T cells in order to stay alive, germinal centers with EZH2-mutant cells appear unaffected even when treated with a CD40L-blocking antibody.[125] This suggest that aberrant repression by EZH2 somehow allows cells to bypass this CD40 stimulation requirement.

While the increased expression of EZH2 has not been shown to lead to lymphomagenesis directly in the absence of other mutations, it does lead to an accelerated rate of tumor growth when combined with other mutations. In transgenic mice carrying a mutated form of EZH2 (specifically, the Y641 mutation mentioned above), blood counts are normal, and there is no difference in lymphoma incidence or overall life expectancy. The only difference is that the proportion of GC B cells in lymphoid follicles is higher in transgenic compared to wild-type mice. In combination with a *MYC* translocation, however, EZH2 mutations lead to increased rates of GC B cell proliferation and B cell lymphomagenesis. Compared to mice with a *MYC* translocation only, mice with simultaneous MYC and EZH2 dysregulation develop tumors earlier. This shows that although EZH2 mutations do not cause lymphoma by themselves, they accelerate their development in the presence of other mutations.[87] Indeed, EZH2 gain-of-function mutations have been reported in one third

of all B cell lymphomas with *MYC* translocations. Similarly, BCL2 and EZH2 aberrations also commonly co-occur more frequently than could be explained by simple coincidence.[84,] The same is true of BCL6, which shares many targets with EZH2, and whose dysregulation be required for mutant EZH2 to cause GC hyperplasia at all.[127]

EZH2 has also been shown to be differentially utilized by tumor cells and used to evade detection by $CD8^+$ cytotoxic T cells. It does so by establishing bivalency at genes controlling antigen processing and MHC class I presentation.[128] With the decreased presentation of cancer-specific antigens, tumor cells are protected from T cell-mediated killing. This type of immune evasion is common in cancer in general, and unfortunately also causes resistance to immunotherapy reliant on T cell-antigen interactions.[129,130]

4.2.3. Therapeutic implications

Since lymphomagenesis is greatly supported by EZH2 hyperactivation, the development of EZH2 inhibitors is of great interest. The first inhibitor targeting EZH2, tazemetostat, competes with S-adenosylmethionine for binding to EZH2, and therefore lowers the ability of EZH2 to catalyze methylation. EZH2 inhibition leads to a global decrease in H3K27me3 levels and promotes the expression of several genes that are associated with B cell differentiation, including *PRDM1*.[131] It has also been shown to decrease proliferation rates and induce apoptosis,[132] and increase MHC I expression on tumor cells.[133] In a trial on patients with refractory or relapsed lymphoma, it proved to be safe and effective – more so in patients with mutant EZH2 in comparison to those with the wild-type form.[134]

4.3. CREBBP/EP300

4.3.1. Structure and function

CREBBP and EP300 are structurally and functionally related enzymes that belong to the KAT3 family of acetyl transferases. They show some degree of redundancy[135] but also have important individual roles.[136] They have been found to interact with hundreds of proteins and function as super-regulators of entire transcription networks.[137]

CREBBP/EP300 acetylate all histone proteins, although they have a preference for H3.[47] In particular, they catalyze the acetylation of lysine 27 at histone 3 (H3K27ac). Together with H3K4me1, H3K27ac marks developmental enhancers as active.[91] H3K27ac also allows for the expression of multiple critical genes, such as those induced by BCR or nuclear receptor signaling.[135,138] Apart from their acetyl transferase domains, CREBBP/EP300 also possess a bromodomain – a domain capable of recognizing and binding to existing acetylated lysine residues. This binding then likely activates CREBBP/EP300 at other H3K27 residues in enhancer regions.[139] By helping to regulate gene expression, CREBBP and EP300 play a role in a wide range of processes, including hematopoiesis,[140] skeletal muscle function,[141] and MHC II antigen presentation.[142] They have also been shown to be important in double strand break repair.[48]

4.3.2. Role in lymphomagenesis

In GC-derived lymphoma, mutations of CREBBP/EP300 are usually loss-of-function mutations affecting their histone acetyl transferase (HAT) domain.[68] Interestingly, the exact mutation sites tend to be different between DLBCL and FL.[143] Following a CREBBP/EP300 loss of function, there is a global decline in H3K27ac peaks in lymphoma cells – this decline is mainly seen on enhancers, while marks on promoters remain largely unaffected.[144] Similarly to KMT2D and EZH2, CREBBP/EP300 loss probably needs to occur as an early event to support lymphomagenesis.[145]

In normal germinal center B cells, both CREBBP and EP300 are present at virtually all super-enhancers in high numbers. These include regulatory elements that control the proto-oncogene *MYC*, genes involved in the regulation of the GC reaction (such as *MEF2B – Myocyte Enhancer Factor 2B)*, or the regulation of MHC class II antigen presentation (such as *CIITA – Class II Major Histocompatibility Complex Transactivator)*.[144,146] Overall, the main function of CREBBP/EP300 is to allow cells to exit the GC reaction and become terminally differentiated. For instance, they bind to the enhancers controlling *PRDM1* and *IRF4*, both of which are positive regulators of GC B cell differentiation.[146] When a loss-of-function mutation is introduced, these genes are repressed and cells are unable to exit the GC.[144] Moreover, in the GCs of

CREBBP-deficient mice, there are fewer plasmablasts despite these cells proliferating more readily upon stimulation with anti-CD40 and IL-4.[146] This shows that without CREBBP (and likely also EP300), GC B cells do not differentiate into plasmablasts, and instead remain dividing in the GC.

Despite this, a loss of CREBBP/EP300 alone is usually not sufficient to cause lymphoma. In combination with BCL2 dysregulation, however, it has been shown to have a very strong pro-lymphoma effect – as many as 92% of experimental mice with both aberrations developed the disease.[146] Additionally, tumors with both aberrations are more aggressive and invasive than those with dysregulated BCL2 only.[144]

Another member of the BCL2 family of proteins, BCL6, is also closely associated with the functions of CREBBP/EP300, as well as the development of GC-derived lymphomas. CREBBP/EP300 and BCL6 generally work in opposition – while CREBBP/EP300 activate transcription, BCL6 acts as a transcriptional repressor, largely by recruiting histone deacetylases (HDACs). Together, they regulate the expression of genes like the components of the BCR, BCL2, and p53.[146] This means that if one side of this balance gets dysregulated, the other takes over and aberrantly represses/activates genes involved in various pathologies. During the GC reaction, the effect of BCL6 is greater – the repression it causes allows cells to maintain the GC phenotype. When CREBBP/EP300 are upregulated, they acetylate BCL6, which lowers its ability to act as a repressor,[146] and consequently leads to the activation of genes required for cells to exit the GC reaction and differentiate. In cells with mutated CREBBP/EP300, BCL6 maintains its repressive functions. Since it works by recruiting HDACs, such as HDAC3, these tumors become dependent on them. In fact, when HDAC3 is knocked down, the balance between CREBBP/EP300 and BCL6 seems to be restored to a certain degree – these cells demonstrate decreased levels of proliferation and increased levels of apoptosis and MHC II antigen presentation.[144]

The importance of CREBBP/EP300 for antigen presentation has been mentioned previously. Indeed, CREBBP-deficient tumor B cells have been shown to have a lowered expression of many MHC class II genes. This is likely because mutant CREBBP fails to acetylate the enhancers regulating *CIITA*, which is a positive master regulator of MHC II expression. As a result, T cells present in the tumor microenvironment receive less stimulation and have decreased rates of proliferation.[67] This phenomenon is especially pronounced in tumors with co-occurring CREBBP and KMT2D mutations, which are severely deficient in cytotoxic $CD8^+$ T cells.[147] This defect in antigen presentation likely allows cancer cells to escape from T cell-mediated killing, and has been associated with worse clinical outcomes.[148] Similarly, CREBBP/EP300 loss also causes immune evasion by leading to the downregulation of ligands normally recognized by natural killer (NK) cells, and thus protects the tumor from being recognized and destroyed.[149]

The tumor microenvironment can also be altered by CREBBP/EP300 loss in other ways. An over-activation of the NOTCH signaling pathway has been observed in lymphoma patients with CREBBP/EP300

mutations. This is due to the decreased expression of the pathway's suppressor caused by CREBBP/EP300 loss. An over-activated NOTCH pathway drives the increased expression of chemokines that recruit M2 phenotype immunosuppressive macrophages to the tumor site – further supporting immune system evasion.[150,151]

It is important to note that CREBBP/EP300 may also promote tumorigenesis by other means – such as the acetylation of non-histone proteins. In some instances, CREBBP/EP300 regulate non-histone proteins that are themselves involved in epigenetic regulation. Such is the case with DOT1L (Disruptor of telomeric silencing 1-like), which is an enzyme that catalyzes H3K79 methylation, and which can be acetylated by CREBBP, and, to a lesser extent, by EP300. Upon acetylation, DOT1L becomes more stable, and its levels subsequently increase. This is significant since high DOT1L levels have been associated with various cancers.[152]

Another example of non-epigenetic CREBBP/EP300 is its acetylation and the resulting activation of prostaglandin I2 synthase, an enzyme involved in prostacyclin synthesis.[153] Since prostacyclin supports regulatory T cell (Treg) differentiation, and lower numbers of Tregs in lymphoma are associated with worse prognosis,[154] this might be another way in which CREBBP/EP300 loss facilitates tumor growth.

4.3.4. Therapeutic implications

The most promising therapies targeting CREBBP/EP300 in lymphoma are those based on histone deacetylase (HDAC) inhibitors, usually combined with standard chemotherapy or radiation. As mentioned previously, GC-derived tumors that have mutated CREBBP/EP300 usually become dependent on HDACs, since they allow them to silence genes that would otherwise cause GC exit and differentiation. Therefore, inhibiting HDACs might at least slow down tumor progression, if not stop it entirely.[155] The most extensively tested of these inhibitors is vorinostat, which has been shown to induce lymphoma cell death and to support the effects of standard chemotherapeutic drugs, both *in vitro* and *in vivo*.[156,157]

As mentioned previously, CREBBP-deficient tumors frequently have a lower MHC II expression, leading to lower levels of (among others) tumor-infiltrating cytotoxic T cells. Related therapies, therefore, focus on improving natural anti-cancer immunity. One example of this is the use of Toll-like receptor (TLR) agonists. The injection of a TLR9 agonist directly into the tumor microenvironment has been shown to overcome this immune evasion strategy and lead to a decreased tumor burden in patients with follicular lymphoma.[158]

5. Conclusion

In recent years, many publications have drawn attention to the role of epigenetic modifiers in tumorigenesis. Lymphomas, particularly certain subtypes of B cell lymphomas derived from germinal center B cells, are no exception to this. While they do often possess other cancer-typical mutations (such as in genes coding proteins involved in cell cycle checkpoints or the DNA damage response), mutations in genes coding for epigenetic modifiers have been found to be particularly common and almost a hallmark of these lymphoma subtypes.

KMT2D, EZH2, CREBBP, and EP300 are among the most frequently mutated histone-modifying enzymes in lymphomas of GC origin. In a broad sense, their mutations fall into of of two categories: 1) loss-of-function-mutations, seen in KMT2D, CREBBP, and EP300, and 2) gain-of-function mutations, seen in EZH2. The first three enzymes are functionally related and serve the same overall function – to activate genes that cause GC B cells to exit the GC reaction with the consequent differentiation into either memory B cells or antibody-secreting plasma cells. EZH2, on the other hand, normally silences these sets of genes and has to be downregulated in order for B cells to exit the GC. This explains the different general types of lymphoma mutations associated with each enzyme's function.

The group of genes regulated by a relatively small number of histone-modifying enzymes is quite large and goes beyond the regulators of B cell differentiation (e.g. *PRDM1*, *IRF4*). Some other targets include oncogenes (e.g. *MYC*, *BCL6*), tumor suppressor genes (e.g. *TP53*, *CDKN1A*), components of the CD40 and NF-κB signaling pathways, or genes regulating antigen presentation.

Importantly, somatic mutations do not occur in isolation in any kind of cancer. The same is true for lymphomas affected by alterations in histone-modifying enzymes. There is a broad spectrum of mutations that work together with these mutant enzymes to promote lymphoma development, the growth of tumor cells, and their potential invasion and dissemination into other tissues. Examples of other frequent lymphoma-associated mutations are cell-cycle checkpoint regulators (such as *CDKN1A*), or genes that help regulate apoptosis (such as those in the *BCL2* family).

Future research may hopefully provide further insight into the mechanisms that these enzymes employ to regulate the GC reaction – for example, how are they recruited to specific genomic loci, and how they cooperate with other proteins, particularly those of the BCL2 family. Overall, the main issue with the dysregulation of histone-modifying enzymes is the normally precisely regulated balance between activating and repressive epigenetic marks on enhancers and promoters, which is incredibly important during certain stages of the GC reaction. This balance, when tipped in the wrong direction at the wrong time, can substantially contribute to the complex process ultimately leading to tumorigenesis. And as evidenced by the use of HDAC inhibitors in CREBBP/EP300 mutant lymphomas, restoring this balance can hold promising therapeutic potential.

6. References

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